On peroxidase activity of Mammalian Glutathione Peroxidase

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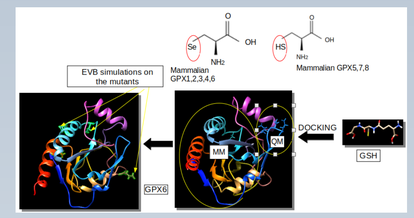
ABSTRACT

The biological effects of selenium are largely mediated by selenium-containing proteins (selenoproteins) [4]. Different isoforms of Glutathione Peroxidase (GPX) have been labeled as potential targets to control or cause the oxidative stress during cancer evolution. [5] [6] In particular, eight different cysteine and selenocysteine containing isoforms of Glutathione Peroxidase (GPX1-8) isoforms have been identified in humans [7]. The relationships between the mechanism and the structure is not entirely understood, although it has been shown that catalytic activity of several reconstructed ancestral structures of GPX6 recover their oxidative activity when the active site is mutated from Cys to Sec. All this results have led us to propose using a combination of QM/MM, empirical valence bond (EVB) calculations and structural bioinformatics tools to unravel sequence-structure-function relationships in GPX6 and its orthologs.



Central Question :
GPX6 appears to lose it's peroxidase activity when there is substitution of S in place of Se, does this loss happen only due to this switching in the active site or the accumulation of mutations play a significant role in the process ?

HYPOTHESIS

There appears to exist an evolutionary shift from Sec to Cys-containing sequences in the glutathione peroxidase protein. Due to this shift, the peroxidase activity is lost in GPX6 when Se is replaced with S. We hypothesize that the loss may be correlated with the accumulation of mutations

OBJECTIVE

DONE: To explore the mode of interaction between GPX6 and the glutathione cofactor in ancestral to modern GPX6.

IN PROGRESS: To understand The enzymatic mechanism of GPX6 and its variants.

FUTURE: To create a model that integrates bioinformatics, computational biochemistry tools and a machine learning models to propose the most likely evolutionary pathway by GPX6.

INTRODUCTION

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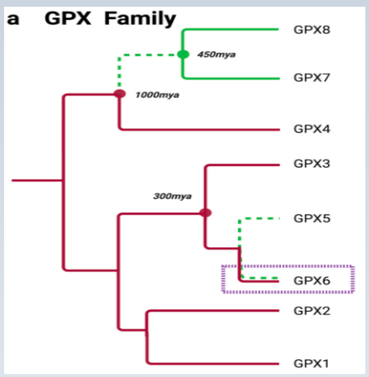

Fig 1a. Topology of the phylogeny of the Eumuroida GPX6Cys clade, green branches, with the Jerboa GPX6Sec lineage red branch and the basal Eumuroida lineage, dashed green branch [3]

1b

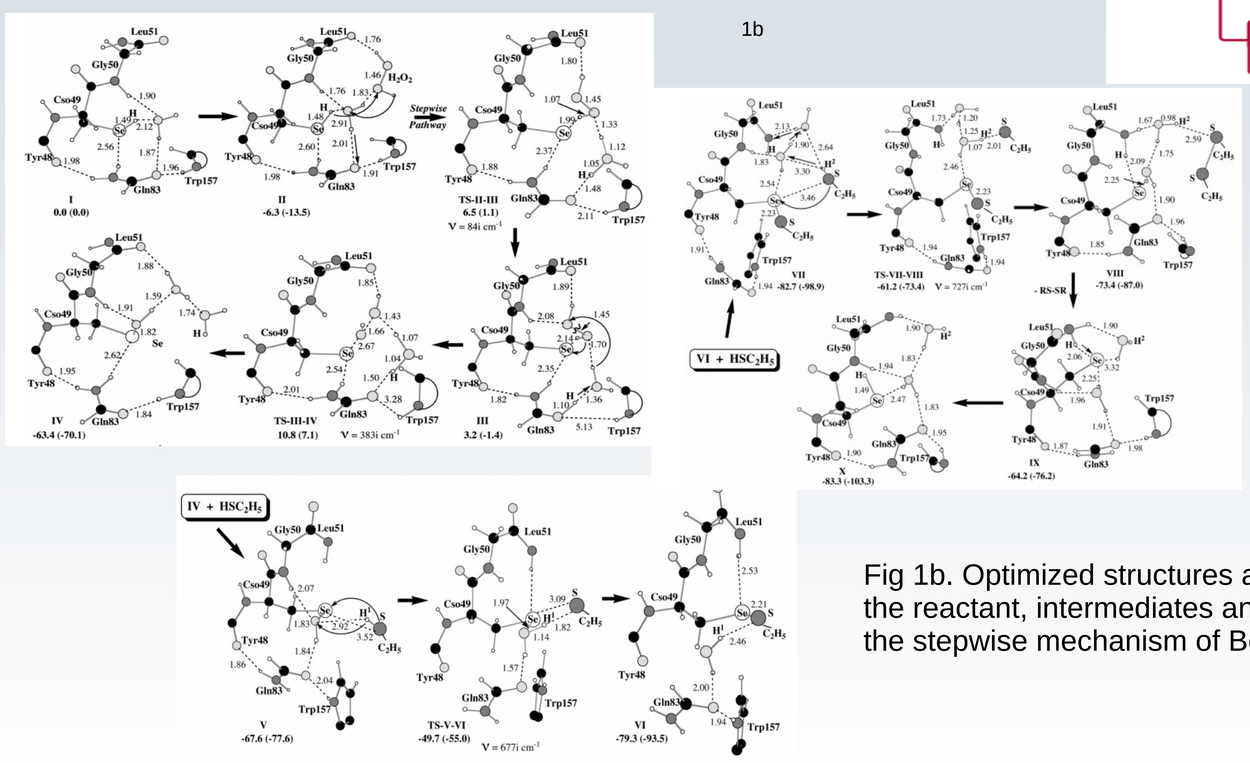

Fig 1b. Optimized structures and energies in kcal/mol] of the reactant, intermediates and TS for the stepwise mechanism of Bovine GPX [2]

Step 1 – RSeH (selenol) + H_2O_2 → RSeOH (selenenic acid) + H_2O

Step 2 - RSeOH + GSH → GS-SeR (selenenyl sulfide) + H_2O

Step 3 - GS-SeR + GSH (glutathione) → GS-SG + RSeH (glutathione disulfide)

RESULTS AND CONCLUSION

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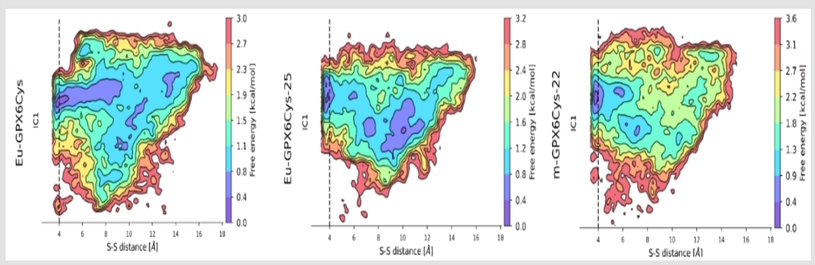

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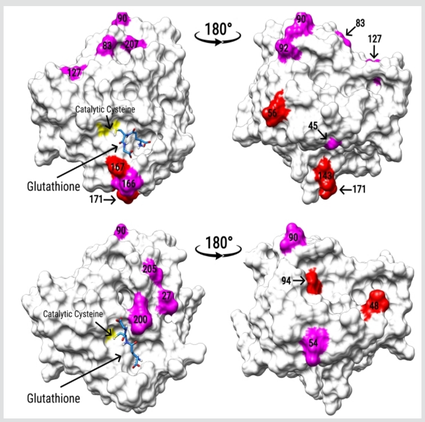

Computational analysis suggests that the binding of GSH and overall structures of the enzymes have not been adversely affected by the involvement of Cys.The conservation of GPX6-glutathione interactions, despite the loss of peroxidase activity maysuggest additional functional roles of GPX6 still to be described.

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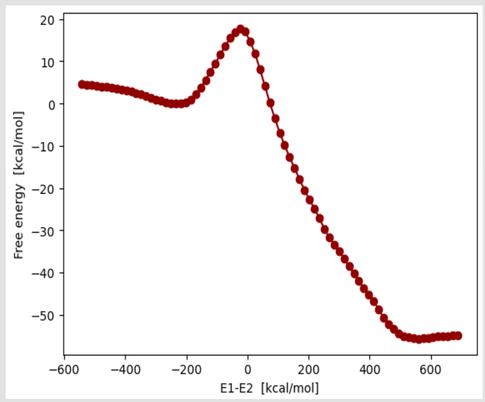

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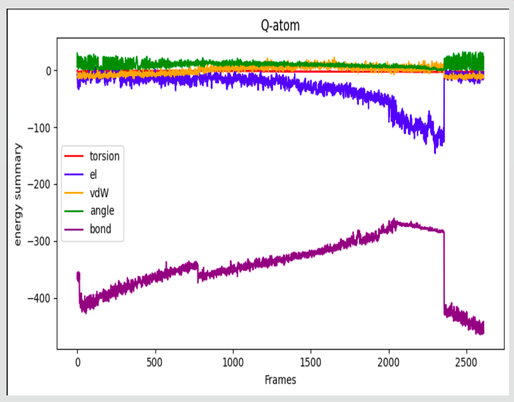

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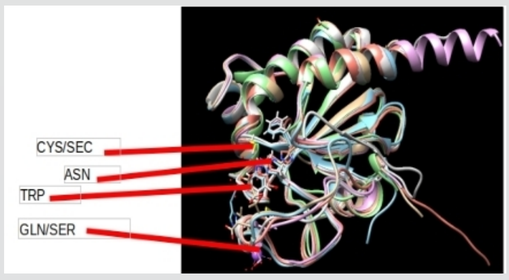

Fig 2- Free energy profiles for the docking of the glutathione dimer to ancestral and modern GPX6 Cys proteins.
Fig 3- Convergence patterns from Eu-GPX6Cys to Eu-GPX6Cys+25 (top) and from Eu-GPX6Cys+25 to m-GPX6Cys+22 (Mouse-GPX6) (bottom). The catalytic cysteine (yellow) is shown with the glutathione best binding energy conformation.
Fig 4- Free energy profile from EVB simulations of reaction 1 of Mammalian wild type GPX6
Fig 5- Energy summary of q-atoms after FEP calculation
Fig 6-Structural homology in Mammalian GPX showing the catalytic residues

FURTHER WORK

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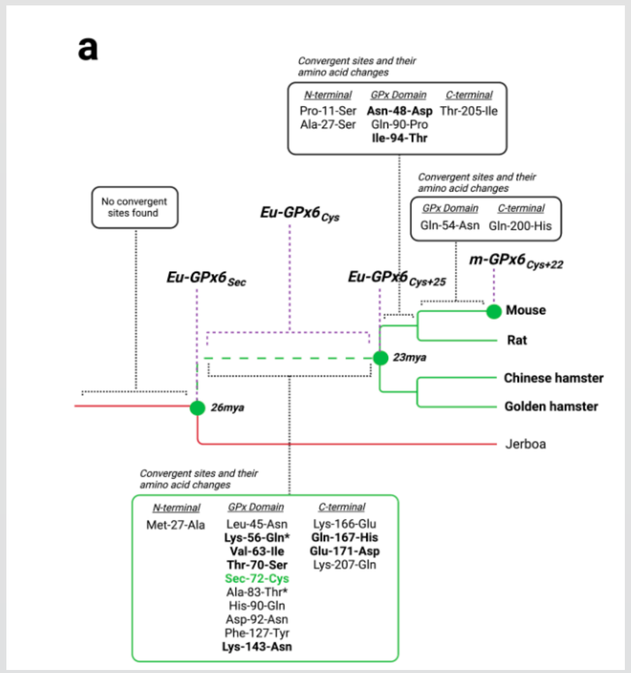

Fig 7a. QM/MM methods to unravel the reaction mechanism in modern Cys- and Sec containing GPX6. The above preliminary results show a well defined path of variants. [3] So, our next goal is now to run EVB simulations with high convergence confidence on the different mutants. For which we will use the Q6 program.

METHODS

1.Alphafold2 for protein structure reconstruction from ancestral sequences.
2.Molecular dynamics simulations (OpenMM, AMBER ff) to explore the conformational landscapes of GPX6 and its complexes with glutathione disulfide
3.Protein-ligand binding energy landscape explorations was done using the PELE software (Protein Energy Landscape Exploration)
4.Q6 program to run EVB simulations and plot the activation energy graph and energy summary plot

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